

REMARKS

The Office Action of January 27, 2000 presents the examination of claims 6-9, 12 and 13. Claims 6-9, 12, and 13 are amended. No new matter is inserted into the application.

Claim Objections

The Examiner objects to claim 6 for being dependent from non-elected claims. Applicants amend claim 6 into independent form. Thus, the instant objection is overcome.

Rejection under 35 U.S.C. § 112, second paragraph

Claims 6-9 and 12-13 are rejected under 35 U.S.C. § 112, second paragraph, for allegedly being indefinite. Applicants respectfully traverse. Reconsideration of the claims and withdrawal of the instant rejection are respectfully requested.

A. The Examiner asserts that the phrase "capable of yielding" in claims 6-9 and 12-13 is not clear. In response to the Examiner's remarks, Applicants amend the phrase to "yields." Thus, the instant rejection is overcome.

B. The Examiner asserts that the phrase "through intracellular decomposition" in claims 6-9 and 12-13 is not clear. In response to the Examiner's remarks, Applicants amend the phrase to "through intracellular processing." The term "processing" is

widely used in the art and therefore comports with the statute. As evidence thereof, Applicants submit the following journal article as Exhibit 1 (attached hereto): *Nature Medicine*, 1:1140 (1995). See, for example, page 1140, left column, second full paragraph, line 12. It is well known in the art that peptides are processed and presented on MHC molecules. As recited in MPEP 2173.02 (page 2100-146), "Definiteness of claim language must be analyzed not in a vacuum, but in light of ... the claim interpretation that would be given by one possessing the ordinary level of skill in the pertinent art." For these reasons, one of skill in the art can clearly determine the scope of the claims. As such, the instant rejection is overcome.

C. The Examiner asserts that the phrase "functionally equivalent properties" in claims 7-8 and 12-13 is unclear. In response to the Examiner's remarks, Applicants delete said phrase. Thus, the instant rejection is overcome.

D. The Examiner asserts that the phrases "derivative" and "derivate" in claims 7-8 and 12-13 is indefinite. In response to the Examiner's remarks, Applicants delete these phrases. Thus, the instant rejection is overcome.

E. The Examiner rejects the phrase "a tumor antigen produced by the expression." In response to the Examiner's remarks, Applicants delete said phrase. Thus, the instant rejection is

overcome.

F. The Examiner rejects the phrase "stringent conditions" in claims 6-9 and 12-13. In response to the Examiner's remarks, Applicants amend the phrase to "stringent conditions comprising 6xSSC, 50% formamide, and 0.5% SDS and a temperature of 42⁰C" as disclosed on page 11, lines 17-19 of the instant specification. Thus, the instant rejection is overcome.

G. The Examiner rejects claims 12-13 for the use of the term "medicine." In response to the Examiner's remarks, Applicants amend the term to "composition," as suggested by the Examiner. Thus, the instant rejection is overcome.

Applicants respectfully submit that the above amendments render the instant claims in full compliance with 35 U.S.C. § 112, second paragraph. Thus, Applicants respectfully request that the instant rejection applied to the claims is withdrawn.

Issues under 35 U.S.C. § 112, First Paragraph

The Examiner rejects claims 6-9 and 12-13 under 35 U.S.C. § 112, first paragraph, for allegedly being not enabled by the instant specification. Applicants respectfully traverse. Reconsideration of the claims and withdrawal of the instant rejection are respectfully requested.

First, the Examiner asserts that the specification does not provide enablement for "any variant protein of SEQ ID NO:2 which contains any unspecified number of substitutions, deletions, additions, or derivatives." In response to the Examiner's remarks, the claims are amended so that "any variant protein of SEQ ID NO:2" is not claimed. Thus, the instant rejection is overcome.

Second, the Examiner asserts that the specification does not provide enablement for any "variant DNA which hybridizes to the DNA of SEQ ID NO:1 under any stringent conditions." In response to the Examiner's remarks, the claims are amended so that "any stringent conditions" is not claimed. Specifically, the claims now recite the stringent hybridization conditions of 6xSSC, 50% formamide, and 0.5% SDS and a temperature of 42⁰C (as disclosed on page 11, lines 17-19 of the instant specification). It is noted that these hybridization conditions are substantially the same as those recited in Example 9 of the USPTO Revised Interim Written Description Guidelines Training Materials. Further, one of the most famous manuals used in the art, *Molecular Cloning, a Laboratory Manual* (attached hereto as Exhibit 2), states that standard hybridization conditions routinely used in the art is 68⁰C in aqueous solution or 42⁰C in 50% formamide (see page 324, line 6). Finally, the instant specification specifically provides the hybridization conditions in Examples 2 and 3. Thus, the instant rejection is overcome.

Third, the Examiner rejects claims 12 and 13 for being "broadly drawn to a medicine..." In response to the Examiner's remarks, Applicants amend the term to "composition," as suggested by the Examiner. Thus, the instant rejection is overcome.

For all of the above reasons, Applicants respectfully submit that all of the instant claims comply with 35 U.S.C. § 112, first paragraph, and respectfully request that the instant rejection be withdrawn.

Rejection Under 35 U.S.C. § 102

The Examiner rejects claims 6-9 and 12-13 under 35 U.S.C. § 102(b) for allegedly being anticipated by Nakao et al. (*Cancer Res.* 55:4248-4252). Applicants respectfully traverse. Reconsideration of the claims and withdrawal of the instant rejection are respectfully requested.

Nakao et al. discloses a peptide antigen expressed on SCC from KE-4 tumor cells. The Examiner asserts that the peptide antigens disclosed by Nakao et al. are the same peptide antigens of the present invention, absent evidence to the contrary.

In order to overcome this rejection, Applicants submit herewith a Declaration executed by Dr. Itoh (first named Inventor) demonstrating that the claimed peptide antigens are distinct from those disclosed by Nakao et al. A signed Declaration will follow. First, however, Applicants note that there are plural tumor antigen

proteins expressed on KE-4 and recognized by KE-4CTL, and, in addition, the active fraction No. 23 of Fig. 3 on page 4251 of Nakao et al. does not necessarily indicate that the fraction contains any isolated tumor antigen peptide or protein, much less the tumor antigen protein/peptide of the present invention. Thus, the possibility that Nakao et al. even discloses a single isolated tumor antigen peptide has not been established.

Nevertheless, the Declaration attached hereto shows that the inventive tumor antigen protein/peptide was only capable of isolation and identification through the technique of expression cloning (see 5.1.3.). It is known in the art that HPLC, when conducted under the conditions recited in Nakao et al., results in peaks containing plural peptides. In other words, the active peak (No. 23) corresponds to a mixture of peptides of unknown structure. Thus, such a disclosure of multiple peptides of unknown structure clearly does not anticipate an isolated tumor antigen protein or peptide of known structure (see 5.2.4.).

For the above reasons, Applicants respectfully submit that Nakao et al. fails to anticipate the present invention. Withdrawal of the instant rejection is therefore requested.

The Examiner rejects claims 6-8 and 12-13 under 35 U.S.C. § 102(e) for allegedly being anticipated by Tsui '667 (U.S.P. 5,776,667). Applicants respectfully traverse. Reconsideration of

the claims and withdrawal of the instant rejection are respectfully requested.

The Examiner writes, "Because the claims recite all or part of the amino acid sequence of 785-793, the art of Tsui et al. reads on the claims." In response to the Examiner's remarks, Applicants amend claims 7 and 8 to delete the phrase "all or part." Thus, the instant claims do not read on Tsui '667, and the instant rejection is overcome.

The Examiner rejects claims 6-9 and 12-13 under 35 U.S.C. § 102(e) for allegedly being anticipated by Boon et al. (*J. Exp. Med.* 183:725-729, 1996). Applicants respectfully traverse. Reconsideration of the claims and withdrawal of the instant rejection are respectfully requested.

The Examiner asserts, "The protein of Boon et al. is a variant of the claimed protein and peptides and it would be inherent that the Boon et al. protein would contain peptides that would also bind to MHC class I antigen and be recognized by T cells." Applicants respectfully disagree. According to the journal article by Nicolina Renkvist, et al., *Cancer Immunol. Immunother.* (2001) 50:3-15 (attached hereto as Exhibit 3) that provides the list and classification of tumor antigen peptides identified so far, the peptide of the present invention (named "SART-1") and that of Boon et al. (named "MAGE") are divided into different groups. See page

5, Table 1 and page 7, Table 3 of Renkvist et al. These Tables show that these peptides are utterly different in terms of classification, origin, distribution of expression, and structure. Accordingly, Boon et al. fails to anticipate the present invention as claimed. Withdrawal of the rejection is therefore respectfully requested.

Overall, the present invention possesses significant patentable features that the cited prior art references do not possess. Furthermore, Applicants submit that amendments to the instant claims render them fully in compliance with 35 U.S.C. § 112, first and second paragraphs. All of the present claims define patentable subject matter such that this application should be placed into condition for allowance. Early and favorable action on the merits of the present application is thereby requested.

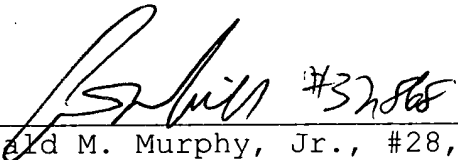
If there are any minor matters precluding allowance of the present application which may be resolved by a telephone discussion, the Examiner is respectfully requested to contact Kristi L. Rupert, Ph.D. (Reg. No. 45,702) at (703) 205-8000.

Pursuant to 37 C.F.R. §§ 1.17 and 1.136(a), Applicant(s) respectfully petition(s) for a two (2) month extension of time for filing a reply in connection with the present application, and the required fee of \$380.00 is attached hereto.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17; particularly, extension of time fees.

Respectfully submitted,

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Attachments: Exhibit 1: STROMINGER, J.L. Nature Med., vol.1 (1995)
Exhibit 2: MANIATIS, T. et al. (1992)
Exhibit 3: RENKVIST, Nicolina et al. (2001)
Declaration Under 37 C.F.R. § 1.132

Version to Show Changes Made:

Claim 6 (Amended)

An isolated [A] tumor antigen protein selected from the group consisting of:

(a) a protein comprising an amino acid sequence shown in SEQ ID NO:1;

(b) a protein encoded by a DNA comprising a nucleotide sequence shown in SEQ ID NO:2; and

(c) a protein encoded by a DNA which hybridizes to a complement of the DNA of SEQ ID NO:2 under stringent hybridization conditions comprising 6xSSC, 50% formamide, and 0.5% SDS and a temperature of 42°C, wherein said protein yields, through intracellular processing, peptide fragment(s) which binds to major histocompatibility complex (MHC) class I antigen and is recognized by cytotoxic T lymphocytes (CTLs) in such binding state [produced by expression of DNA of claim 1 or 2].

Claim 7 (Amended)

An isolated [A] tumor antigen peptide consisting of [comprising] part of the protein of claim 6, which binds [can bind] to MHC class I antigen to be recognized by CTLs [T cells, or a derivative thereof having functionally equivalent properties].

Claim 8 (Twice Amended)

An isolated [A] tumor antigen peptide of claim 7 which comprises [all or part of] the amino acid sequence of positions 749-757, 736-744, 785-793, or 690-698 in the amino acid sequence of SEQ ID NO:1 [SEQ ID NO:2, or a derivative thereof having functionally equivalent properties].

Claim 9 (Amended)

A composition [medicine] comprising, as an active ingredient, the tumor antigen protein of claim 6 [the tumor antigen peptide R derivative thereof defined in claim 7 or 8].

Claim 12 (Amended)

A composition [medicine] comprising, as an active ingredient, the tumor antigen peptide of [or derivate thereof as defined in] claim 7.

Claim 13 (Amended)

A composition [medicine] comprising, as an active ingredient, the tumor antigen peptide of [or derivate thereof as defined in] claim 8.

Peptide vaccination against cancer?

Micrometastases in a murine lung carcinoma model have regressed or been prevented (pages 1179–1183).

The development of our knowledge of antigen processing and presentation, and of techniques for the isolation of MHC molecules and analysis of their bound peptides, presents the opportunity to solve problems of great medical importance. These include efforts to blockade, anergize or delete T cells that mediate autoimmune diseases, as well as efforts to enhance immunity to infectious agents and tumours. A striking application to the prevention of metastatic disease in the murine Lewis lung carcinoma model is described in this issue of *Nature Medicine*¹.

The possibility of immunotherapy for human tumours is offered by the facts that some tumours (particularly melanoma and renal carcinoma) rarely undergo spontaneous remission and that low-level immune responses against some other tumours have been detected. These and other facts have led to much research aimed at identifying both tumour-specific antigens that can stimulate immune responses and the peptides from the processed antigens that are presented to the immune system by MHC molecules. In many cases, these tumour antigens are normal self-proteins with a limited cell/tissue distribution, but they may also occur in the tumour in a mutated form or be derived from virus-associated cancers. In these latter cases they would behave immunologically as foreign proteins/peptides, although self peptides can also induce immune responses.

Several genes encoding tumour antigens that may give rise to low-level immune responses in experimental animals and man have been described^{2–4}. In addition, several peptide epitopes, presented by class I MHC molecules to T cells derived from tumour hosts, have been identified. These include, notably, mutant peptide(s) derived from the gap junction protein connexin-37 found in the murine Lewis lung carcinoma⁵ and peptides from several different human melanoma proteins^{6–9}. In addition, nine peptides from the oncogenic E6 and E7 proteins of human papilloma virus associated with cervical carcinoma that bind to HLA-A2 were identified. Four of these induced a vigorous cytotoxic T-lymphocyte (CTL) response in HLA-A2 transgenic mice, and human CTL generated with these same peptides could lyse an

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HLA-A2 cervical carcinoma cell line¹⁰. Many other class I restricted peptides have also been under study^{2–4}. However, in addition to class I-MHC-restricted CD8⁺ T-cells, class II MHC-restricted CD4⁺ T-cells can also mediate a cytolytic response¹¹, a fact of particular importance in the tissue destruction in some class II-associated autoimmune diseases. Notably, therefore, a peptide of 25 residues presented by a murine class II MHC protein has been identified that is associated with an immune response against a murine tumour¹².

Can such peptides be utilized in the therapy of tumours? Therapeutic goals might include prophylaxis against some tumours (vaccination), prevention of metastatic spread after surgical removal of tumours or resolution of the tumour mass itself. Several reports addressing these questions have appeared and many more studies must be in progress. For example, vaccination has protected against a tumour formed using a transformed mouse cell line¹³, and established tumours in the mouse have also been eradicated using CTL raised against the same peptide¹⁴. Vaccination trials in humans have been initiated. Remarkably, in the present study¹, metastatic spread from a primary tumour mass that was allowed to develop for 30 days before surgical excision and vaccination has been prevented. If these animal studies can be extended to examples of human tumours, then a window may have opened on a rational approach to the immunotherapy of at least a few human cancers.

The mode of administration of peptides is of critical importance in such trials, because soluble peptide is thought to induce tolerance (and thus may be important in the treatment of autoimmune diseases). In the present animal studies¹ which were designed to enhance immunity, peptide was administered in incomplete Freund's adjuvant, but in human trials various other techniques may be employed. For example, peptide pulsed onto autologous antigen presenting (dendritic) cells, encapsulated in liposomes or modified as lipopeptides have all been explored. DNA vaccination

simultaneous use of cytokines that may enhance immunogenicity and the linkage of CTL epitopes to helper T-cell epitopes are among other factors that must be considered. Finally, despite all efforts, tumour escape variants may constitute a major problem. Generalized loss of expression of MHC antigens or allele-specific loss are thought to represent major problems in the immune surveillance of tumours. The road ahead is unpaved and full of potholes, but the journey could be rewarding.

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2. Boon, T., Certottini, J., van den Eynde, B., van der Bruggen, P. & Van Pel, A. Tumor antigens recognized by T lymphocytes. *Annu. Rev. Immun.* 12, 337–365 (1994).
3. Nanda, N. & Sercarz, E. Induction of anti-self-immunity to cure cancer. *Cell* 82, 13–17 (1995).
4. Moller, G. Tumor immunology. *Immunol. Rev.* 145, 5–250 (1995).
5. Mandelboim, O. *et al.* CTL induction by a tumour associated antigen octapeptide derived from a murine lung carcinoma. *Nature* 369, 67–71 (1994).
6. Cox, A. *et al.* Identification of a peptide recognized by five melanoma-specific human cytotoxic T cell lines. *Science* 264, 716–719 (1994).
7. Castelli, C. *et al.* Mass spectrometric identification of a naturally processed melanoma peptide recognized by CD8⁺ cytotoxic T lymphocytes. *J. exp. Med.* 181, 363–368 (1995).
8. Van den Eynde, B. *et al.* A new family of genes coding for an antigen recognized by autologous cytolytic T lymphocytes on a human melanoma. *J. exp. Med.* 182, 689–698 (1995).
9. Wolfel, T. *et al.* A p16-INK4a-insensitive CDK4 mutant targeted by cytolytic T lymphocytes in a human melanoma. *Science* 269, 1281–1284 (1995).
10. Rensing, M. *et al.* Human CTL epitopes encoded by human papillomavirus type 16 E6 and E7 identified through in vivo and in vitro immunogenicity studies of HLA-A*0201-binding peptides. *J. Immun.* 154, 5934–5943 (1995).
11. Krensky, A., Reiss, C., Mier, J., Strominger, J., Burakoff, S. Long-term human cytolytic T-cell lines allospecific for HLA-DR6 antigen are OKT4⁺. *Proc. natl. Acad. Sci. U.S.A.* 79, 2365–2369 (1982).
12. Monach, P., Meredith, S., Siegel, C. & Schreiber, H. A unique tumor antigen produced by a single amino acid substitution. *Immunity* 2, 45–49 (1995).
13. Melief, C. & Kast, M. T-cell immunotherapy of tumors by adoptive transfer of cytotoxic T lymphocytes and by vaccination with minimal essential epitopes. *Immunol. Rev.* 146, 167–177 (1995).
14. Feltkamp, M. *et al.* Cytotoxic T lymphocytes raised against a subdominant epitope offered as a synthetic peptide eradicate human papillomavirus type 16-induced tumors. *Eur. J. Immunol.* 25, 2638–2642 (1995).

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Molecular Cloning

A LABORATORY MANUAL

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Cold Spring Harbor Laboratory
1982

HYBRIDIZATION OF DNA OR RNA IMMOBILIZED ON FILTERS TO RADIOACTIVE PROBES

There are many methods available to hybridize radioactive probes in solution to DNA or RNA immobilized on nitrocellulose filters. These methods differ in the following aspects:

- the solvent and temperature used (68°C in aqueous solution or 42°C in 50% formamide);
- the volume of solvent and the length of hybridization (large volumes for periods as long as 3 days or minimal volumes for times as short as 4 hours);
- the degree and method of agitation (continuous shaking or stationary);
- the concentration of the labeled probe and its specific activity;
- the use of compounds, such as dextran sulfate, that increase the rate of reassociation of nucleic acids;
- the stringency of washing following the hybridization.

Although the choice depends to a large extent on personal preference, we would like to offer the following guidelines.

1. Hybridization reactions in 50% formamide at 42°C are easier to set up, present less of an evaporation problem, and are less harsh on the filters than is hybridization at 68°C in an aqueous solution. The kinetics of the hybridization reaction in 80% formamide are approximately three to four times slower than in an aqueous solution (Casey and Davidson 1977). Assuming a linear relationship between the rate of hybridization and formamide concentration, the rate in 50% formamide should be two times slower than in an aqueous solution.
2. The smaller the volume of hybridization solvent, the better. The kinetics of nucleic acid reassociation are faster, and the amount of probe needed may be reduced so that the DNA on the filter acts as the driver for the reaction. All these are important parameters when detecting clones of low-abundance mRNAs. However, it is essential that sufficient liquid be present for the filters to remain at all times covered by a film of the hybridization solution.
3. Continual movement of the probe solution across the filter is unnecessary, even for a reaction driven by DNA immobilized on the filter. However, if a large number of filters are hybridized simultaneously, agitation is advisable in order to prevent the filters from adhering to each other.
4. The kinetics of the hybridization reaction are difficult to predict from theoretical considerations, partly because the exact concentration of the immobilized nucleic acid and its availability for hybridization are unknown.

When using probes made by nick translation of double-stranded DNA, the following rule of thumb is useful: Allow the hybridization to proceed for a time sufficient to enable the probe in solution to achieve $1-3 \times C_0t_{1/2}$. In 10 ml of hybridization solution, 1 μ g of a probe of 5-kb complexity will reach $C_0t_{1/2}$ in 2 hours. To determine the time of half-renaturation for any other probe, simply enter the appropriate values into the following equation:

$$\frac{1}{X} \times \frac{Y}{5} \times \frac{Z}{10} \times 2 = \text{number of hours to achieve } C_0t_{1/2}$$

where,

X = the weight of probe added (in μ g)

Y = its complexity (for most probes, complexity is proportional to the length of the probe in kb)

Z = the volume of the reaction (in ml)

After hybridization for $3 \times C_0t_{1/2}$ has been reached, the amount of the probe available for additional hybridization to the filter is negligible. For single-stranded cDNA probes, the hybridization time may be shortened since the lack of a competing DNA strand in solution favors hybridization to DNA bound to the filter.

5. In the presence of dextran sulfate, the rate of association of nucleic acids is accelerated because the nucleic acids are excluded from the volume of the solution occupied by the polymer. Their effective concentration is therefore increased. The rate of association reportedly increases 10-fold in the presence of 10% dextran sulfate (Wahl et al. 1979).

Although dextran sulfate is useful in circumstances where the rate of hybridization is the limiting factor in detecting sequences of interest, it is unnecessary for most purposes. It is also difficult to handle because of its viscosity and sometimes can lead to high backgrounds.

6. In general, the washing conditions should be as stringent as possible; i.e., a combination of temperature and salt concentration should be chosen that is slightly (5°C) below the T_m of the hybrid under study. The temperature and salt conditions can often be determined empirically in preliminary experiments where Southern blots (see pages 382ff) of genomic DNA are hybridized to the probe of interest and then washed under conditions of different stringency.

REVIEW

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A listing of human tumor antigens recognized by T cells

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Key words Antigens · Tumor · T cells · Epitopes

Complete list of abbreviations of tumor antigens 707-AP 707 alanine proline · AFP *alpha* (α)-fetoprotein · ART-4 adenocarcinoma antigen recognized by T cells 4 · BAGE B antigen · β -catenin/*m* β -catenin/mutated · Bcr-abl breakpoint cluster region–Abelson · CAMEL CTL-recognized antigen on melanoma · CAP-1 carcino-embryonic antigen peptide-1 · CASP-8 caspase-8 · CDC27*m* cell-division-cycle 27 mutated · CDK4/*m* cyclin-dependent kinase 4 mutated · CEA carcino-embryonic antigen · CT cancer/testis (antigen) · Cyp-B cyclophilin B · DAM differentiation antigen melanoma (the epitopes of DAM-6 and DAM-10 are equivalent; but the gene sequences are different; DAM-6 is also called MAGE-B2, and DAM-10 is also called MAGE-B1) · ELF2*M* elongation factor 2 mutated · ETV6-AML1 Ets variant gene 6/acute myeloid leukemia 1 gene ETS · G250 glycoprotein 250 · GAGE G antigen · GnT-V N-acetylglucosaminyltransferase V · Gp100 glycoprotein 100 kDa · HAGE helicase antigen · HER-2/*neu* human epidermal receptor-2/neurological · HLA-A*0201-R170I arginine (R) to isoleucine (I) exchange at residue 170 of the α -helix of the α 2-domain in the HLA-A2 gene · HPV-E7 human papilloma virus E7 · HSP70-2*M* heat shock protein 70-2 mutated · HST-2 human signet ring tumor-2 · hTERT or hTRT human telomerase reverse transcriptase · iCE intestinal carboxyl esterase · KIAA0205 name of the gene as it appears in databases · LAGE L antigen · LDLR/FUT low-density lipid receptor/GDP-L-fucose: β -D-galactosidase 2- α -L-fucosyltransferase ·

MAGE melanoma antigen · MART-1/Melan-A melanoma antigen recognized by T cells-1/melanoma antigen A · MC1R melanocortin 1 receptor · Myosin/*m* myosin mutated · MUC1 mucin 1 · MUM-1, -2, -3 melanoma ubiquitous mutated 1, 2, 3 · NA88-A NA cDNA clone of patient M88 · NY-ESO-1 New York-esophagus 1 · P15 protein 15 · p190 minor bcr-abl protein of 190 kDa bcr-abl · Pml/RAR α promyelocytic leukaemia/retinoic acid receptor α · PRAME preferentially expressed antigen of melanoma · PSA prostate-specific antigen · PSM prostate-specific membrane antigen · RAGE renal antigen · RU1 or RU2 renal ubiquitous 1 or 2 · SAGE sarcoma antigen · SART-1 or SART-3 squamous antigen rejecting tumor 1 or 3 · TEL/AML1 translocation Ets-family leukemia/acute myeloid leukemia 1 · TPI/*m* triosephosphate isomerase mutated · TRP-1 tyrosinase related protein 1, or gp75 · TRP-2 tyrosinase related protein 2 · TRP-2/INT2 TRP-2/intron 2 · WT1 Wilms' tumor gene

Abbreviations used ALL acute lymphoblastic leukemia · AML acute myeloid leukemia · APL acute promyelocytic leukemia · CML chronic myelogenous leukemia · CTL cytotoxic T lymphocytes · Ets E-26 transforming specific (family of transcription factors) · H/N head and neck · MHC major histocompatibility complex · NSCLC non-small cell lung carcinoma · ORF open reading frame · RCC renal cell carcinoma · SCC squamous cell carcinoma · TSTA tumor-specific transplantation antigens

Introduction

Since the cloning of *MAGE-1* [125], the first gene reported to encode a human tumor antigen recognized by T cells, molecular identification and characterization of tumor antigens has mainly been achieved for melanoma. A major reason for this lies in the difficulty of establishing cell lines in vitro from other types of cancer, such lines being necessary to generate tumor-specific CTL

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lines or clones to be used in the genetic or biochemical approach aimed at molecularly identifying new cancer antigens. More recently, however, new approaches have allowed the discovery of new antigens recognized by T cells even in tumors other than melanoma.

It is, then, important to categorize these antigens, particularly for the HLA allele restricting their recognition by T cells and for their tissue distribution. With this purpose, tumor antigens have been collected in the present work and briefly commented.

The list presented in the tables below includes all T-cell-defined epitopes encoded by tumor antigens and published by 31 July 2000. Analogs or artificially modified epitopes are excluded from the list. Only tumor antigens recognized by T cells (either cytotoxic CD8+ or helper CD4+) are listed, given their potential importance in the control of tumor growth. Other antigens, identified by antibodies, are excluded but a large collection of them, as detected by the Serex technology, can be found in the data base of the Institute for Cancer Research (www.licr.org/SEREX.htm). It is of note that many tumor antigens (e.g. *MAGE*, NY-ESO-1a) are now known to be recognized by both T cells and antibodies in the same cancer patients [54].

In the tables herein, tumor antigens are listed in alphabetic order along with the epitope sequence and the HLA allele which restricts recognition by T cells. Furthermore, data on the tissue distribution of each antigen are provided, making this listing an important source for easily retrieving data concerning human tumor antigens.

The listing is meant to be a tool for scientists and students who have an interest in the field of tumor immunology and immunotherapy. The bibliography allows a rapid search for more detailed information at the single antigen or epitope level.

We do not ignore, however, the fact that by recent technologies (e.g., subtractive hybridization, representational-difference analysis, microarrays) hundreds of genes are being detected which are preferentially expressed or overexpressed in neoplastic cells as compared with normal counterparts or are expressed in metastatic but not in primary, early lesions (e.g., melanoma, breast cancer, lymphoma). By using appropriate computer algorithms [9], a number of new epitopes will be identified that can bind MHC molecules. By applying such approaches, a large array of gene products can be screened for their potential antigenic function. More cumbersome may be the selection of the most immunogenic epitopes through appropriate functional assays.

Classification of tumor antigens

Group 1: Class I HLA-restricted cancer/testis antigens (Table 1)

A milestone in tumor immunology was certainly the cloning of *MAGE-1* [125] and the subsequent characterization of the first T-cell-defined antigenic epitope a

year later [119]. Those findings were rapidly followed by the identification of new members within this group [6, 123]. The *MAGE*, *BAGE* and *GAGE* families of genes were born. The antigens belonging to this group, now including also NY-ESO-1, were called cancer/testis (CT) antigens for their expression in histologically different human tumors and, among normal tissues, in spermatocytes/spermatogonia of testis and, occasionally, in placenta. These antigens now represent one of the main components for antitumor vaccine development. CT antigens result from reactivation of genes normally silent in adult tissues [27], but that are transcriptionally activated in some tumors [30]. Their expression in testis does not provide targets for an immune reaction because cells of testis do not express class I HLA [56]. Despite the fact that the CT genes are probably the most characterized ones, their physiological function remains largely unknown.

Considering that new genes in the group of CT antigens have been cloned (CT9 [105], CT10 [46], LAGE [72], *MAGE-B5*, *-B6*, *-C2*, *-C3* and *-D* [74, 75], *HAGE*, *SAGE* [80]), but that no T-cell epitopes have been identified from them yet, the question arises as to how many more genes encoding CT antigens remain to be discovered and how many epitopes exist that could be of use in cancer immunotherapy.

Group 2: Class I HLA-restricted differentiation antigens (Table 2)

These antigens are shared between tumors and the normal tissue from which the tumor arose; most are found in melanomas and normal melanocytes [2]. Many of these melanocyte lineage-related proteins are involved in the biosynthesis of melanin. Epitopes recognized by both CD8+ and CD4+ T cells can be derived from melanosome proteins [8, 118, 135, 136].

Group 3: Class I HLA-restricted widely expressed antigens (Table 3)

Genes encoding widely expressed tumor antigens have been detected in many normal tissues as well as in histologically different types of tumors with no preferential expression on a certain type of cancer. It is possible that the many epitopes expressed on normal tissues are below the threshold level for T-cell recognition, while their overexpression in tumor cells can trigger an anticancer response even by breaking a previously established tolerance. These widely expressed gene products have revealed a broad spectrum of mechanisms that are involved in generating T-cell-defined epitopes through alterations in gene transcription and translation. To highlight some examples, the epitope of CEA is derived from a non-AUG-defined alternative ORF [1], while the RU2 gene creates its epitope by reverse strand transcription [124].

Table 1 Class I HLA-restricted cancer/testis antigens. All these antigens were found to be expressed by normal spermatocytes and/or spermatogonia of testis. Occasionally *MAGE-3*, *MAGE-4* and

the *GAGE* genes were found to be expressed also in placenta [26, 24]. The NY-ESO-1 antigen was found to be expressed in normal ovary cells [18]

Gene	HLA allele	Peptide epitope	Authors [ref.]	Tissue distribution among tumors ^a
<i>MAGE-A1</i>	A1	EADPTGHSY	Traversari et al. 1992 [119]	Melanoma, breast carcinoma, SCLC [27, 29, 125] – sarcoma, NSCLC [27, 29] – thyroid
<i>MAGE-A1</i>	A3	SLFRAVITK	Chaux et al. 1999a [16]	medullary carcinoma [125] – colon
<i>MAGE-A1</i>	A24	NYKHCFPEI	Fujie et al. 1999 [37]	carcinoma [27] – laryngeal tumors [29]
<i>MAGE-A1</i>	A28	EVYDGREHSA	Chaux et al. 1999a [16]	
<i>MAGE-A1</i> , -A2, -A3, -A6	B37	REPVTKAEML	Tanzarella et al. 1999 [113]	Melanoma, colon and breast carcinomas, SCLC [27, 29, 125] – sarcoma, NSCLC [27, 29] – thyroid medullary carcinoma, H/N tumors, bronchial SCC [125] – laryngeal tumors [29] – leukemias [27]
<i>MAGE-A1</i>	B53	DPARYEFLW	Chaux et al. 1999a [16]	Melanoma, breast carcinoma, SCLC [27, 29, 124] – sarcoma, colon carcinoma, NSCLC [27, 29] – thyroid medullary carcinoma [125]
<i>MAGE-A1</i>	Cw2	SAFPTTINF	Chaux et al. 1999a [16]	
<i>MAGE-A1</i>	Cw3	SAYGEPRKL	Chaux et al. 1999a [16]	
<i>MAGE-A1</i>	Cw16	SAYGEPRKL	van der Bruggen et al. 1994b [127]	
<i>MAGE-A2</i>	A2	KMVELVHFL	Visseren et al. 1997 [128]	Melanoma, colon and breast carcinomas, SCLC [27, 29, 125] – sarcoma, NSCLC [27, 29] – thyroid medullary carcinoma [125] – laryngeal tumors [77] – leukemias [29]
<i>MAGE-A2</i>	A2	YLQLVFGIEV	Visseren et al. 1997 [128]	
<i>MAGE-A2</i>	A24	EYLQLVFGI	Tahara et al. 1999 [110]	
<i>MAGE-A3</i>	A1	EADPIGHLY	Gaugler et al. 1994 [40]	Melanoma, colon and breast carcinomas [27, 125] – H/N tumors [18] – bronchial
<i>MAGE-A3</i>	A2	FLWGPRALV	van der Bruggen et al. 1994a [126]	SCC, thyroid medullary and bladder carcinoma, sarcomas, SCLC, NSCLC [125] – leukemias [29]
<i>MAGE-A3</i>	A24	TFPDLESEF	Oiso et al. 1999 [89]	
<i>MAGE-A3</i>	A24	IMPKAGLLI	Tanaka et al. 1997 [111]	
<i>MAGE-A3</i>	B44	MEVDPIGHLY	Herman et al. 1996 [48], Fleischhauer et al. 1996 [35]	
<i>MAGE-A3</i>	B52	WQYFFPVIF	Russo et al. 2000 [103]	
<i>MAGE-A4</i>	A2	GVYDGREHTV	Duffour et al. 1999 [33]	Melanoma, NSCLC, sarcomas, esophageal, colon and breast carcinomas [27]
<i>MAGE-A6</i>	A34	MVKISGGPR	Zorn and Hercend, 1999b [147]	Melanoma, NSCLC, colon carcinoma, leukemias [27]
<i>MAGE-A10</i>	A2	GLYDGMHL	Huang et al. 1999 [52]	Not defined
<i>MAGE-A12</i>	Cw7	VRIGHLYIL	Panelli et al. 2000 [91], Heidecker et al. 2000 [47]	Melanoma, myeloma, brain tumors, sarcoma, leukemias, SCLC, NSCLC, H/N tumors, bladder, lung, esophageal, breast, prostate and colorectal carcinoma [27]
<i>BAGE</i>	Cw16	AARAVFLAL	Boël et al. 1995 [6]	Melanoma, bladder and mammary carcinomas, H/N SCC, NSCLC, sarcoma
<i>DAM-6, -10</i>	A2	FLWGPRAYA	Fleischhauer et al. 1998 [36]	Melanoma, skin tumors, mammary and ovarian carcinomas [77] – lung carcinoma [25, 77] – seminomas [25]
<i>GAGE-1, -2, -8</i>	Cw6	YRPRPRRY	Van den Eynde et al. 1995 [123], De Backer et al. 1999 [26]	Melanoma, sarcoma, NSCLC, SCLC, mesothelioma, sarcoma, seminoma, leukemias, lymphomas, H/N tumors, bladder, esophageal, mammary, colon, prostate carcinomas
<i>GAGE-3, -4, -5, -6, -7B</i>	A29	YYWPRPRRY	De Backer et al. 1999 [26]	Melanomas, H/N tumors, leukemias, esophageal, lung and bladder carcinomas
<i>NA88-A</i>	B13	MTQGQHFLQKV	Moreau-Aubry et al. 2000 [82]	Melanoma
<i>NY-ESO-1</i>	A2	SLLMWITQCFL	Jäger et al. 1998 [54]	Melanoma, sarcoma, B-lymphomas, hepatoma, H/N tumors, bladder, lung, prostate, ovarian, thyroid and breast carcinoma [18]
<i>NY-ESO-1a</i> (<i>CAG-3</i>)	A2	SLLMWITQC	Jäger et al. 1998 [54]	
	A2	QLSLLMWIT	Jäger et al. 1998 [54]	
	A31	ASGPGGGAPR	Wang et al. 1998b [134]	

^a Tissue distribution among tumors as described in the given references when different from the paper first reporting the sequence of the epitope

Group 4: Class I HLA-restricted, tumor-specific antigens (Table 4)

Unique tumor antigens arise from point mutations of normal genes (like β -catenin, CDK4) [98, 137], whose molecular changes often accompany neoplastic trans-

formation or progression. These antigens are thus expressed only in the individual tumor where they were identified, since it is unlikely that the same mutation may occur in two different neoplasms unless it involves genes (e.g. RAS) whose alteration is an obligatory step in neoplastic transformation.

Table 2 Class I HLA-restricted melanocyte differentiation antigens. These antigens can only be expressed in normal and neoplastic cells of the same lineage (namely melanocytes, skin, retina, peripheral ganglia) or in normal cells of the prostate gland

Gene	HLA allele	Peptide epitope	Authors [ref.]
<i>MART-1/Melan-A^a</i>	A2	AAGIGILTV	Coulie et al. 1994 [22], Kawakami et al. 1994a [58]
	A2	EAAGIGILTV	Schneider et al. 1998 [106]
	A2	ILTVILGVL	Castelli et al. 1995 [14]
	B45	AEEAAGIGIL	Schneider et al. 1998 [106]
	B45	AEEAAGIGILT	Schneider et al. 1998 [106]
<i>MCIR</i>	A2	TILLGIFFL	Salazar-Onfray et al. 1997 [104]
	A2	FLALIICNA	Salazar-Onfray et al. 1997 [104]
<i>Gp100</i>	A2	KTWGQYWQV	Bakker et al. 1995 [3]
	A2	AMLGTHTMEV	Tsai et al. 1997 [120]
	A2	MLGTHTMEV	Tsai et al. 1997 [120]
	A2	SLADTNSLAV	Tsai et al. 1997 [120]
	A2	ITDQVPFSV	Kawakami et al. 1995 [61]
	A2	LLDGTATLRL	Kawakami et al. 1994b [59]
	A2	YLEPGPVTA	Cox et al. 1994 [24]
	A2	VLYRYGSFSV	Kawakami et al. 1995 [61]
	A2	RLMKQDFSV	Kawakami et al. 1998 [62]
	A2	RLPRIFCSC	Kawakami et al. 1998 [62]
	A3	LIYRRRLMK	Kawakami et al. 1998 [62]
	A3	ALNFPQSQK	Kawashima et al. 1998 [65]
	A3	SLIYRRRLMK	Kawashima et al. 1998 [65]
	A3	ALLAVGATK	Skipper et al. 1996 [108]
	A24	VYFFLPDHL	Robbins et al. 1997 [99]
	Cw8	SNDGPTLI	Castelli et al. 1999 [15]
<i>PSA</i>	A1	VSHSFPHPLY	Corman et al. 1998 [20]
	A2	FLTPKKLQCV	Correale et al. 1997 [21]
	A2	VISNDVCAQV	Correale et al. 1997 [21]
<i>PSM Tyrosinase</i>	A1	HSTNGVTRIY	Corman et al. 1998 [20]
	A1	KCDICTDEY	Kittlesen et al. 1998 [68]
	A1	SSDYVIPIGTY	Kawakami et al. 1998 [62]
	A2	YMDGTMSQV	Wölfel et al. 1994 [137]
	A2	MLLAVLYCL	Wölfel et al. 1994 [137]
	A24	AFLPWHRLF	Kang et al. 1995 [57]
	B44	SEIWRDIDF	Brichard et al. 1996 [10]
<i>TRP-1 (or gp75)</i>	A31	MSLQRQFLR	Wang et al. 1996b [132]
<i>TRP-2</i>	A2	SVYDFFVWL	Parkhurst et al. 1998 [92]
	A2	TLDSQVMSL	Noppen et al. 2000 [86]
	A31	LLGPGRPYR	Wang et al. 1996a [131]
	A33	LLGPGRPYR	Wang et al. 1998a [133]
	Cw8	ANDPIFVVL	Castelli et al. 1999 [15]

^a Two different groups simultaneously discovered this gene and gave it two different names, MART-1 and Melan-A respectively

Table 3 Class I HLA-restricted widely expressed antigens

Gene	HLA	Peptide epitope	Tissue distribution		Reference
			Tumors	Normal tissues	
<i>ART-4</i>	A24	AFLRHAAL DYPSLSATDI	SCC, SCLC, H/N tumors, leukemia, lung, esophageal, gastric, cervical, endometrial, ovarian and breast carcinomas	Testis, placenta, fetal liver	Kawano et al. 2000 [64]
<i>CAMEL</i>	A2	MLMAQEALAFI	Melanoma	Testis, placenta, heart, skeletal muscle, pancreas	Aarnoudse et al. 1999 [1]
<i>CEA</i>	A2	YLSGANLNL (CAP-1) ^a	Melanoma	Testis, placenta, heart, skeletal muscle, pancreas	Tsang et al. 1995 [121]
<i>CEA</i>	A3	HLFGYSWYK	Colon, rectum, pancreas, gastric, breast and lung carcinomas	Gastrointestinal embryonic tissue	Kawashima et al. 1999 [66]
<i>Cyp-B</i>	A24	KFHRVIKDF DFMIQGGDF	Lung adenocarcinoma, T cell leukemia, lymphosarcoma – bladder, ovarian, uterine and esophageal SCC	Ubiquitously expressed in normal tissues	Gomi et al. 1999 [42]

Table 3 (Continued)

Gene	HLA	Peptide epitope	Tissue distribution		Reference
			Tumors	Normal tissues	
<i>HER2/neu</i>	A2	KIFGSLAFL	Melanoma – ovarian and breast carcinomas	Epithelial cells	Fisk et al. 1995 [34]
<i>HER2/neu</i>	A2	IISAVVGIL	Melanoma, ovarian, pancreatic [96] ^b and breast carcinomas	Epithelial cells	Peoples et al. 1995 [95]
<i>HER2/neu</i>	A2	RLLQETELV	Melanoma, ovarian, gastric, pancreatic [96] and breast carcinomas	Epithelial cells	Kono et al. 1998 [71]
<i>HER2/neu</i>	A2	VVLGVVFGI ILHNGAYSL	Melanoma, ovarian, gastric, pancreatic [96] and breast carcinomas	Epithelial cells	Rongcun et al. 1999 [101]
<i>HER2/neu</i>	A3	YMIMVKCWM VLRENTSPK	Melanoma, ovarian, gastric, pancreatic [96] and breast carcinomas	Epithelial cells	Kawashima et al. 1999 [66]
<i>hTERT^c</i>	A2	ILAKFLHWL	Lung and ovarian carcinomas – multiple myeloma, melanoma, sarcoma, acute leukemias, non-Hodgkin's lymphomas	Hematopoietic stem cells and progenitors; germinal center cells; basal keratinocytes; gonadal cells; certain proliferating epithelial cells	Vonderheide et al. 1999 [131]
<i>hTERT^c</i>	A2	ILAKFLHWL RLVDDFLV	Lung, prostate and ovarian carcinomas, multiple myeloma, melanoma, sarcoma, acute leukemias, non-Hodgkin's lymphomas	Circulating B cells; germinal center B cells; thymocytes; CD34+ progenitor hemopoietic cells	Minev et al. 2000 [81]
<i>ICE</i>	B7	SPRWVPTCL	RCC	Kidney, colon, small intestine, liver, heart, pituitary gland, adrenal gland, prostate, stomach	Ronsin et al. 1999 [102]
<i>MUC1</i>	A11	STAPPAHGV	Breast and ovarian carcinomas, multiple myeloma, B-cell lymphoma	None ^d	Domenech et al. 1995 [31]
<i>MUC1</i>	A2	STAPPVHNV	Breast and ovarian carcinoma, multiple myeloma, B-cell lymphoma	None ^d	Brossart et al. 1999 [11]
<i>MUC2</i>	A2	LLNQLQVNL MLWGWREHV	Ovary, pancreas and breast mucinous tumors, colon carcinoma of non-mucinous type	Colon, small intestine, bronchus, cervix and gall bladder	Böhm et al. 1998 [7]
<i>PRAME</i>	A24	LYVDSLFFL	Melanoma, H/N and lung SCC, NSCLC [122], RCC, adenocarcinoma, sarcoma, leukemias [122]	Testis, endometrium, ovary, adrenals, kidney, brain, skin	Ikeda et al. 1997 [53]
<i>P15</i>	A24	AYGLDFYIL	Melanoma	Testis, spleen, thymus, liver, kidney, adrenal tissue, lung tissue, retinal tissue	Robbins et al. 1995 [97]
<i>RUI</i>	B51	VPYGSFKHV	Melanoma, renal and bladder carcinomas	Testis, kidney, heart, skin, brain, ovary, liver, lung, lymphocytes, thymus, fibroblasts	Morel et al. 2000 [83]
	B7	LPRWPPQQL	Melanoma, sarcomas leukemia – brain, esophageal and H/N tumors – renal, colon, thyroid, mammary, bladder, prostatic and lung carcinomas	Testis, kidney, liver, urinary bladder	Van den Eynde et al. 1999 [124]
<i>SART-1</i>	A24	EYRGFTQDF	Esophageal, H/N and lung SCC – adenocarcinoma, uterine cancer	Testis, fetal liver	Kikuchi et al. 1999 [67]
<i>SART-1</i>	A*2601	KGSGKMKTE	Esophageal, H/N and lung SCC, adenocarcinoma, uterine cancer	Testis, fetal liver	Shichijo et al. 1998 [107]
<i>SART-3</i>	A24	VYDYNCHVDL AYIDFEMKI	H/N, esophageal and lung SCC, adenocarcinoma, leukemia, melanoma	Lymphoid cells, fibroblasts, testis, fetal liver	Yang et al. 1999 [139]
<i>WT1</i>	A2	RMFPNAPYL	Gastric, colon, lung, breast, ovary, uterine, thyroid and hepatocellular carcinomas – leukemia (including AML, ALL and CML)	Kidney, ovary, testis, spleen	Oka et al. 2000 [90]

^a CAP-1 is an alternative name of this peptide^b Tissue distribution among tumors as described in the given references when different from the paper first reporting the sequence of the epitope^c Telomerase is expressed in most human tumors: those listed were shown to be susceptible to lysis by cytotoxic T lymphocytes^d All epithelial tissues express mucin-like hyperglycosylated molecules

Table 4 Class I HLA-restricted tumor-specific antigens, including both unique (CDK-4, MUM-1, MUM-2, β -catenin, HLA-A2-R170I, ELF2 m, myosin-m, caspase-8, KIAA0205, HSP70-2m) and shared (CAMEL, TRP-2/INT2, GnT-V, G 250) antigens

Gene	HLA allele	Peptide epitope	Tissue distribution		Reference
			Tumors	Normal tissues ^a	
<i>AFP</i>	A2	GVALQTMKQ	Hepatocellular carcinoma	Fetal liver	Butterfield et al. 1999 [12]
<i>β-Catenin/m</i>	A24	SYLDSGIHF	Melanoma	None	Robbins et al. 1996 [98]
<i>Caspase-8/m</i>	B35	FPSDSWCYF	H/N tumors	None	Mandruzzato et al. 1997 [78]
<i>CDK-4/m</i>	A2	ACDPHSGHFV	Melanoma	None	Wölfel et al. 1995 [138]
<i>ELF2 M</i>	A68	ETVSEQSNV	Lung SCC	None	Hogan et al. 1998 [50]
<i>GnT-V</i>	A2	VLPDVFIRC(V) ^a	Melanoma, brain tumors, sarcoma	Breast and brain (low expression)	Guilloux et al. 1996 [45]
<i>G250</i>	A2	HLSTAFARV	RCC, colon, ovarian and cervical carcinomas	None	Vissers et al. 1999 [129]
<i>HSP70-2M</i>	A2	SLFEGIDIY	RCC, melanoma, neuroblastoma	None	Gaudin et al. 1999 [39]
<i>HA-A*0201-R170I</i>	A2	CVEWLRIYLENGK	RCC	None	Brändle et al. 1996 [9]
<i>HST-2</i>	A31	YSWMDISCWI	Gastric signet cell carcinoma	None	Suzuki et al. 1999 [109]
<i>KIAA0205</i>	B44*03	AEPINIQTV	Bladder cancer	None	Gueguen et al. 1998 [44]
<i>MUM-1</i>	B44	EEKLIVVLF	Melanoma	None	Coulie et al. 1995 [23]
<i>MUM-2</i>	B44	SELFRSGLDY	Melanoma	None	Chiari et al. 1999 [19]
<i>MUM-2</i>	Cw6	FRSGLDSYV	Melanoma	None	Chiari et al. 1999 [19]
<i>MUM-3</i>	A28	EAFIQPITR	Melanoma	None	Baurain et al. 2000 [4]
<i>Myosin/m</i>	A3	KINKNPKEYK	Melanoma	None	Zorn and Hercend, 1999a [146]
<i>RAGE</i>	B7	SPSSNRIRNT	Melanoma, sarcomas, mesotheliomas, H/N tumors, bladder, renal, colon and mammary carcinomas	Retina only	Gaugler et al. 1996 [41]
<i>SART-2</i>	A24	DYSARWNEI	H/N and lung SCC, lung adenocarcinoma, RCC, melanoma, brain tumors, esophageal and uterine cancers	None	Nakao et al. 2000 [85]
		AYDFLYNYL			
		SYTRLFLIL			
<i>TRP-2/INT2</i>	A68	EVISCKLIKR	Melanoma	None	Lupetti et al. 1998 [76]
<i>707-AP</i>	A2	RVAALARDA	Melanoma	None ^b	Morioka et al. 1995 [84]

^a VLPDVFIRC(V) = nonamer and decamer peptides are both recognized by CTLs

^b This antigen is not expressed in normal cells but, as the tissue of the testis was not tested, it will not become clear to which category the antigen may belong until more information is available

In mouse models unique antigens have been shown to be more immunogenic than the other groups of shared antigens [32]; since unique antigens are responsible for the rejection of tumor transplants in mice, they have been defined as tumor-specific transplantation antigens (TSTA). The unique antigens are the most specific targets for immunotherapy, but this potential advantage must be balanced against the almost total impossibility of their clinical use, as they can induce an immune response only against the original tumor on which they were found.

Other tumor-specific but shared antigens have been described which are generated by alteration in splicing mechanisms and which occur in tumor but not in normal cells, as in the case of TRP-2/INT2 [76].

Group 5: Class II HLA-restricted antigens (Table 5)

Stimulation of the CD4⁺ T helper cells by tumor antigens is considered to be impaired or absent in

cancer patients and this may be the reason of an insufficient immune response to tumors. Therefore the identification of tumor antigen epitopes recognized by such lymphocytes is a crucial step in the long sought improvement of antitumor immune response that may result into clinical efficacy. The first epitope presented by a class II HLA and capable of provoking a CD4⁺ T-cell response was identified in 1994 in melanoma tyrosinase [117]. Then a gap of 4 years followed during which only one additional epitope was characterized [118], before other genes encoding class II-restricted peptides were discovered. However, as the technical and methodological approaches for identifying CD4⁺ T-cell epitopes of tumor antigens have become available, an exponential increase in reporting such epitopes has been seen. In fact, since 1998 as many as 27 new class II HLA-restricted epitopes from 14 antigens have been molecularly identified using, among others, II-cDNA fusion libraries [135], immunized transgenic mice [145] and biochemical approaches [96].

Table 5 Class II HLA-restricted antigens

Gene	HLA allele	Peptide epitope	Tissue distribution		Reference
			Tumors	Normal tissues	
Epitopes from normal protein antigens					
<i>Annexin II</i>	DRB*0401	DVPKWISIM- TERSVPH	Melanoma	Not done	Li et al. 1998 [73]
<i>Gp100</i>	DRB1*0401	WNRQLYPE- WTEAQRLD	Melanoma	Melanocytes	Li et al. 1998 [73]
<i>MAGE-1, -2, -3, -6</i>	DRB*1301 DRB*1302	LLKYRAREP- VTKAE	Melanoma, lung and breast carcinomas, H/N SCC	Testis, placenta	Chaux et al. 1999a [16]
<i>MAGE-3</i>	DR*1101	TSYVKVLHHM- VKISG	Melanoma, lung and breast carcinomas, H/N SCC	Testis, placenta	Manici et al. 1999 [79]
<i>MAGE-3</i>	DRB*1301 DRB*1302	AELVHFLLLK- YRAR	Melanoma, lung and breast carcinomas, H/N SCC	Testis, placenta	Chaux et al. 1999b [17]
<i>MART-1/Melan-A</i>	DRB1*0401	RNGYRALMDKS- LHVGTOCALTRR	Melanoma	Melanocytes	Zarour et al. 2000 [143]
<i>MUC1</i>	DR3	PGSTAPPAHGV	Breast and ovarian cancers, multiple myeloma, B-cell lymphoma	None ^a	Hiltbold et al. 1998 [49]
<i>NY-ESO-1</i>	DRB4*0101	VLLKEFTVSG	Melanoma, B-lymphoma, hepatoma [18] ^b , sarcoma, H/N tumors, – bladder, lung, prostate, ovarian, thyroid and breast carcinomas	Testis	Zeng et al. 2000 [145]
<i>NY-ESO-1</i>	DRB4*0101– 0103	PLPVPGVLLK- EFTVSGNI VLLKEFTVSG- NLTIRLT AADHRQLQL- SISSCLQQL	B-lymphoma, melanoma, sarcoma, H/N tumors, hepatoma [18] – bladder, lung, prostate, ovarian, thyroid and breast carcinomas	Testis	Jäger et al. 2000 [55]
<i>PSA</i>	DR4	ILLGRMSLFM- PEDTG SLFHPEDTGQVFQ QVFQVSHSFPHPLYD NDLMLRLRLSEPAELT KKLQCVQLHVISM GVLQGITSMGSEPCA	Melanoma	Melanocytes	Corman et al. 1998 [20]
<i>Tyrosinase</i>	DRB1*0401	QNILLSNAPLGPQFP DYSYLQSDPD- SFQD SYLQSDSDPDSFQD	Melanoma	Melanocytes	Topalian et al. 1994 [117], Topalian et al. 1996 [118]
<i>Tyrosinase</i>	DRB1*1501	RHRPLQEVYP- EANAPIGHNRE	Melanoma	Melanocytes	Kobayashi et al. 1998a [69]
<i>Tyrosinase</i>	DRB1*0405	EIWRDIDFAHE	Melanoma	Melanocytes	Kobayashi et al. 1998b [70]
Epitopes from mutated protein antigens					
<i>HPV-E7</i>	DR*0401 DR*0407	LFMDTLSEFVCPLC LFMDSLNFEVCPWC	Cervical carcinoma	None	Höhn et al. 1999 [51]
<i>CDC27/m</i>	DRB1*0401	FSWAMDLDPKGA	Melanoma	None	Wang et al. 1999a [135]
<i>TPI/m</i>	DRB1*0101	GELIGILNAAKVPAD	Melanoma	None	Pieper et al. 1999 [96]

^a All epithelial tissues express highly glycosylated mucins whereas tumor cells often show hypoglycosylated mucins with a normal protein sequence

^b Tissue distribution among tumors as described in the given references when different from the paper first reporting the sequence of the epitope

It is of note that even class II-restricted antigens include a subgroup of mutated proteins which, therefore, represent truly tumor-specific antigens.

Group 6: Fusion proteins (Table 6)

In several malignancies, particularly in some forms of leukemia, the molecular mechanism of carcinogenesis

involves translocation of chromosomes which results in fusion of distant genes. This often causes the synthesis of fusion proteins which characterize each type of disease (e.g., bcr-abl and pml-RAR α in CML and APL, respectively) and generate new epitopes that can be recognized by T cells, either CD8⁺ or CD4⁺ in class I or class II HLA restriction, respectively. Although these epitopes appear to be weakly immunogenic in leukemia patients [28], some of these peptides or proteins

Table 6 Epitopes derived from fusion proteins (fusion proteins are never found in normal tissues)

Gene	HLA allele	Peptide epitope	Tissue distribution among tumors	Reference
HLA class I restricted epitopes				
<i>bcr-abl</i> ^a	A2	FMVELVEGA KLSEQESLL MLTNSCVKL	CML	Buzyn et al. 1997 [13]
<i>bcr-abl p210(b3a2)</i>	A2	SSKALQRPV	CML	Yotnda et al. 1998a [141]
<i>bcr-abl (b3a2)</i>	A3	ATGFKQSSK KQSSKALQR	CML	Greco et al. 1996 [43]
<i>bcr-abl p210 (b3a2)</i>	A3, A11	HSATGFKQSSK	CML	Bocchia et al. 1996 [5]
<i>bcr-abl p210(b3a2)</i>	A3	KQSSKALQR	CML	Norbury et al. 2000 [87]
<i>bcr-abl p210(b3a2)</i>	B8	GFKQSSKAL	CML	Norbury et al. 2000 [87]
<i>ETV6/AML</i>	A2	RIAECILGM	ALL	Yotnda et al. 1998b [142]
HLA class II restricted epitopes				
<i>bcr-abl p190 (e1a2)</i>	DRB1*1501	EGAFHGDAEALQRPVAS	ALL	Tanaka et al. 2000 [112]
<i>bcr-abl p210 (b2a2)</i>	DRB5*0101	IPLTINKEEALQRPVAS	CML	ten Bosch et al. 1999 [116]
<i>bcr-abl p210 (b3a2)</i>	DRB1*0401	ATGFKQSSKALQRPVAS	CML	ten Bosch et al. 1996 [115]
<i>bcr-abl p210 (b3a2)</i>	DRB1*1501	ATGFKQSSKALQRPVAS	CML	ten Bosch et al. 1996 [115]
<i>bcr-abl (b3a2)</i>	DRB1*0901	ATGFKQSSKALQRPVAS	CML	Yasukawa et al. 1998 [140]
<i>bcr-abl (b3a2)</i>	DRB1*1101	LIVVIVHSATGFKQSS- KALQRPVA	CML	Pawelec et al. 1996 [93]
<i>bcr-abl (b3a2)</i>	DR11	IVHSATGFKQSSKALQRP- VASDFEP	CML	Bocchia et al. 1996 [5]
<i>Dek-cain</i>	DRB4*0103	TMKQICKKEIRRLHQY	AML	Ohminami et al. 1999 [88]
<i>LDLR/FUT</i>	DRB1*0101	GGAPPVTWRRAPAPG WRRAPAPGAKAMAPG	Melanoma	Wang et al. 1999b [132]
<i>Pml/RARα</i>	DR11	NSNHVASGA- GEAAIETQSSSSEIV [28]	APL	Gambacorti-Passerini et al. 1993 [38]
<i>p190 minor bcr-abl (e1a2)</i>	DRB1*1501	EGAFHGDAEALQRPVAS	AML	Tanaka et al. 2000 [112]
<i>TEL/AML1</i>	DP5, DP17	IGRIAECILGMNPSR	AML	Yun et al. 1999 [143]

^aThese bcr-abl epitopes are not true fusion proteins generated-epitopes, because they derive from outside the bcr-abl junction

Table 7 Frequency of epitopes recognized by a given HLA allele

Antigen	No. of epitopes	HLA-A	HLA-B	HLA-C
MAGE-1, -2, -3, -4, -6, -10, -12	24	13 (54%)	7 (29%)	4 (17%)
GAGE-1, -2, -3, -4, -5, -6, -7B, -8	8	5 (62.5%)	0	3 (37.5%)
MART-1	6	4 (67%)	2 (33%)	0
Gp100	12	11 (92%)	0	1 (8%)
Tyrosinase	6	5 (83%)	1 (17%)	0

can nevertheless be used to pulse dendritic cells for vaccination.

Frequency of epitopes recognized by a given HLA allele (Table 7)

In Table 7 we have summarized, for those antigens from which a high number of epitopes have been described (e.g., CT and differentiation antigens of melanoma) the distribution of epitopes recognized in the context of different HLA loci. This table shows that the majority of epitopes are seen as restricted by HLA-A in all the three groups of antigens considered. Whether this reflects a bias caused by the fact that most of the studies have been carried out with HLA-A-restricted T cells or is mediated by the immunodominant role of the HLA locus in recognition of tumor antigens remains to be established.

Conclusions

Several excellent and timely reviews on tumor antigens have been published periodically during the past few years [8, 63, 100]. However, to our knowledge a comprehensive list of all available tumor antigens and their epitopes and HLA restriction has never been reported, despite the fact that the features of each antigen can be easily found in data bases. We hope that our work may be of interest for many tumor immunologists and students. Needless to say, we may have inadvertently missed information on some antigens despite our careful scrutiny of the published literature; therefore, we will be grateful to any readers who provide us with any missing information. We now plan to update these tables bi-monthly in order to keep our data base as informative as possible. The antigen list can also be found at the INT website (www.istitutotumori.mi.it).

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